

Scientific Advisory Committee on Nutrition

Paper for Discussion: **Food Standards Agency nutrition research programme, the role of dietary lipids in the development of cardiovascular disease, workshops**

Agenda Item 10

The attached paper summarises the discussions, which took place at two workshops organised by the Food Standards Agency:

December 2001: Optimal Dietary Intake of Monounsaturated Fatty Acids (MUFA)

March 2002: Are n-3 Polyunsaturated Fats (PUFA) from plant oils (Alpha-Linolenic Acid or ALA) as beneficial to cardiovascular health as those from fish origin?

Members are asked to consider the attached papers and comment on the conclusions and recommendations of the two workshops.

Scientific Advisory Committee on Nutrition

Reports of Research Workshops

FSA Funded Research Programme: The Role of Dietary Lipids in the Development of Cardiovascular Disease

Background

1. The Food Standards Agency (FSA) is in the process of reviewing its existing research programmes to ensure their current and future policy relevance. To this end, the FSA recently convened a group of expert scientists from academia, industry and health organisations, to evaluate two specific areas of the research programme where groups of projects were coming to an end.
2. To ensure that FSA-funded research was presented in the wider context, results from non-FSA-funded studies were also presented. Internationally recognised experts were appointed to chair two meetings – Prof. Mike Gibney, Trinity College Dublin (December 2001) and Prof. Martijn Katan, Wageningen University, The Netherlands (March 2002).
3. The meeting in December 2001 addressed the issue of defining the optimal dietary intake for monounsaturated fats.
4. The second workshop in March 2002 addressed the issue of whether n-3 polyunsaturated fatty acids (PUFA) from plant oils (alpha-linolenic acid) are as beneficial to cardiovascular health as the n-3 PUFA from fish oils (eicosapentaenoic and docosahexaenoic acid).

Monounsaturated fatty acid workshop

Introduction and terminology

5. A reduction in saturated fat intake lowers the risk of coronary heart disease (CHD) (Clarke *et al*, 1997). Considerable scientific interest has been focussed on how monounsaturated fatty acid (MUFA) intake affects risk for CHD, and specifically on the optimal quantity of MUFA in the diet. This issue has remained unresolved, however, because of uncertainty regarding whether MUFA or carbohydrate (CHO) should be substituted for saturated fatty acids (SFA). Reduction in SFA intake can be achieved either through a decrease in total fat intake, concomitant with a decrease in saturated fat intake, or by replacement of saturated fats with MUFAs or polyunsaturated fatty acids (PUFAs) in the *cis*-isoform.
6. Much of the work in this area has looked at effects on intermediate cardiovascular disease (CVD) risk factors. CHD refers to conditions such as angina, myocardial infarction/heart attack and is a subset of cardiovascular disease, which includes

peripheral vascular disease, such as some types of stroke, dementia and circulatory diseases of the limbs. It is likely that factors which protect against CHD may also be protective against CVD.

7. The seminal work of Keys *et al* (1986) demonstrated a protective effect against CHD of a relatively high fat diet¹, rich in MUFA and low in SFA (7-8% total energy). Since then a body of evidence has accumulated, demonstrating a hypocholesterolaemic effect of the isoenergetic (same energy value) replacement of SFA with MUFA (Mensink & Katan, 1987, 1989; Macdonald *et al*, 1989; Berry *et al*, 1991; Foley *et al*, 1992; Mata *et al*, 1992; Sirtori *et al*, 1992; Warburg *et al*, 1992; Lichtenstein *et al*, 1993; Howard *et al*, 1995; Kris-Etherton *et al*, 1999; Williams *et al*, 1999).
8. A reduction in total and saturated fat intake leads to a decrease in circulating levels of total and LDL-cholesterol serum levels. A similar reduction in total and LDL-cholesterol levels can also be achieved through the isoenergetic replacement of SFA with MUFA. Low fat diets increase serum triacylglycerol (TAG) levels and lower HDL-cholesterol, both of which are associated with increased risk for CHD (Mensink & Katan 1987, 1992; Kris-Etherton *et al* 1999). In contrast, the isoenergetic replacement of SFA with MUFA does not increase TAGs or lower HDL.
9. There has been much discussion as to which is the best approach to reduce SFA intake, and thereby, risk of CVD in the general population. The relative importance of dietary risk factors for CVD in different subpopulations and under different conditions has also been debated (see, Katan *et al*. 1997; Connor & Connor, 1997; Marckmann & Astrup, 2000; Kris-Etherton *et al*, 2000).
10. An increased understanding of the disease processes involved has led to recognition of diet-related risk factors other than circulating cholesterol concentrations. The studies presented at the FSA workshop investigated the effects of isocaloric replacement of SFA with MUFA on haemostasis, postprandial responses, endothelial function and insulin sensitivity as well as on lipoprotein metabolism.

Conclusions and Recommendations

11. It is not clear whether it is better to replace SFA in the diet with MUFA or CHO (i.e. low fat diets). Defining optimal MUFA intake also depends upon defining the optimal total fat intake, which may vary for subgroups of the population (eg: gender, genetic background, presence of obesity, physical activity levels). **Lowering fat intakes implies increases in energy intake from other sources such as carbohydrates: the possible negative impact of a high carbohydrate intake also needs to be considered.** This area was identified as one for future research and discussion.
12. The COMA recommendations (Department of Health, 1994) are for the average contribution of SFA to dietary energy to be no more than about 10% energy and

¹ In Keys' study the range was 33-40% energy

the average contribution of total fat to dietary energy should be no more than 35% energy. The latest figures for the UK, (DEFRA, 2001), show average intakes of total fat are just over 38% energy and total SFA are 15% energy. Progress towards these goals has been slow. **A combined strategy involving both reduction in total fat and the replacement of SFA with *cis*-unsaturated fats may be helpful towards further reducing population cholesterol levels and thus cardiovascular disease risk.**

13. Earlier research concentrated on reductions in SFA through increases in PUFA intakes. Although the beneficial impact of MUFAs on the plasma lipid profile is possibly less than that of n-6 *cis*-PUFAs (Clarke et al. 1997), it is now known that intakes of PUFA greater than 10% energy may have adverse effects on HDL-cholesterol levels (COMA 1994). There are also issues of the greater oxidisability of PUFAs, both in foods during processing and cooking, and in the body, where oxidised lipids are implicated in protein and DNA damage. **Replacement of SFA with MUFA offers an alternative.**
14. Compliance with dietary recommendations remains a problem. **It has been suggested that working with industry to alter directly the food supply may be the most effective population based approach (Schaefer, 2002).**
15. Forty percent (40%) of fat in the diet is from manufactured products. **It may be sensible to encourage the replacement of ‘invisible’ saturated fats with MUFA.** Methods used in the study presented by Christine Williams and previous work (Williams *et al*, 1999; Roche *et al*, 1998) could be employed at the population level: modified oils in catering, in food and spreads manufacture and in the production of ‘healthy’ snacks. Issues such as the higher costs of MUFA relative to SFA and subsequent effects on shelf life would need to be addressed as well as effects on palatability.
16. **There is also the possibility of enabling modification in the fatty acid composition of animal fats and dairy products**, an opportunity that has not been fully realised despite considerable research evidence for feasibility, efficacy and safety, especially for enrichment with MUFA. There would be need to provide industry with clear scientific advice and there may be an issue around incentives.
17. **To provide the scientific basis for such an approach it was recommended that a large multi-centre study should examine the effects on cardiovascular risk of the replacement of saturated fat with monounsaturated fat in relation to varying total fat intakes.** Such a study should be of sufficient size to account for genetic variation, gender, physical activity and stage of life factors; as well as, of sufficient duration to account for adaptation to diets. Because the relative importance of the different risk factors for cardiovascular disease in different populations and under different conditions is much debated, it is important that any study should assess a comprehensive set of risk factors including: endothelial function; insulin sensitivity; lipoprotein metabolism; factor VII and haemostasis; and immune function.
18. **It was also recommended that mechanistic studies should be undertaken to look at the effect of MUFA on:**

- **Metabolism in the gut** – do MUFA inhibit/reduce cholesterol uptake from the gut; is this an effect on chylomicron assembly? What is the role of MUFA in stimulating gut hormone release and the physiological consequences?
 - **Reverse cholesterol transport**
 - **Adipose tissue cytokine release and sCRP release as well as other anti-inflammatory effects** via monocytes
 - **LDL turnover**
19. **The fatty acid composition of the UK diet should be assessed more frequently.** The National Food Survey does not carry this level of detail (only classes of lipids in the diet) so fatty acid analyses of the Total Diet Study (TDS) should be carried out at least every two years. The FSA has addressed this issue raised and intends to commission the latest in a series of analyses of the individual fatty acid composition of the TDS during 2002. However, it is recognised that the TDS is not necessarily the most appropriate means of assessing fatty acid intakes, and other options are currently being considered.

Alpha-linolenic acid workshop

Introduction

20. The long chain n-3 polyunsaturated fatty acids (PUFA), eicosapentaenoic acid (EPA; 20:5 n-3) and docosahexaenoic acid (DHA; 22:6 n-3), have been shown to reduce the recurrence of coronary heart disease (GISSI-Prevenzione Investigators, 1999). EPA and DHA also have a variety of beneficial effects on risk factors for CHD. Oily fish is the richest dietary source of EPA and DHA, but fish consumption is low in habitual UK diets (MAFF, 1997). An alternative source of n-3 PUFA is the more abundant alpha-linolenic acid (ALA; 18:3 n-3), which can be elongated and desaturated to its long chain derivatives EPA and DHA. However, in humans the extent and regulation of this conversion is unclear. The question arises, therefore, whether plant oils rich in ALA (e.g. linseed, rapeseed and nut oils) could have similar beneficial effects to fish oil consumption on reducing the risk of developing cardiovascular diseases, via conversion of ALA to its long chain derivatives.
21. The Lyon Diet Heart Study (de Lorgeril *et al.* 1994, 1999) suggested that dietary ALA may have a remarkably beneficial effect on the secondary prevention of CHD. However, compared to the control diet, the experimental diet was also rich in fruits and vegetables, legumes, fibre-rich cereals, monounsaturated fatty acids, and low in linoleic acid (LA; 18:2 n-6) and saturated fatty acids. This makes it impossible to dissect out the role of individual food components. Several prospective epidemiological studies have reported a protective effect of ALA-rich foods against CHD (Dolecek, 1992; Ascherio *et al.* 1996; Hu *et al.* 1999), although, a more recent study (Oomen *et al.* 2001) of elderly men found no association between ALA intake and CHD.

Effect of ALA supplementation on cardiovascular risk factors

22. The results from studies investigating the effects of ALA supplementation on CHD risk factors have been equivocal. ALA has been shown to be equivalent to LA and n-6-rich oils for lipid and lipoprotein effects (Harris, 1997; Pang *et al.* 1998). Unlike fish oil supplementation, ALA consumption results in no effect on plasma TAG levels (Pang *et al.* 1998).
23. Fish oil supplementation can lead to positive effects on haemostasis (Mutanen & Freese, 2001) while the results for studies investigating any effect of ALA on haemostasis have proved inconclusive (Knapp, 1997; Allman-Farinelli *et al.*, 1999). Arterial endothelial function, a non-invasive marker of arterial health, can be improved by fish oil supplementation (Nestel, 2000), and one study has reported a beneficial effect of a high intake of ALA (20g/day) on endothelial function in obese subjects but with a concomitant reduction in insulin sensitivity and HDL cholesterol (Nestel *et al.* 1997)

Effect of ALA supplementation on markers of inflammation

24. Inflammation is a characteristic of many diseases, including cardiovascular diseases. Fish oil supplementation has been shown to exhibit inhibitory effects on most tests of immune function and on inflammatory markers in humans (Calder, 2001). There are only a few studies of the immune/inflammatory effects of ALA in humans. These show that supplementation with high intakes of ALA (14 and 18 g/day) can partly inhibit lymphocyte proliferation and the production of inflammatory cytokines (Kelley *et al.* 1991; Caughey *et al.* 1996). Studies supplementing with lower amounts of ALA (2 or 4 g/day) reveal limited impact (Healy *et al.* 2000; Thies *et al.* 2001a,b,c).

Conclusions and Recommendations

25. The studies presented as part of this workshop investigate the importance of ALA as a source of long chain n-3 PUFA and its influence on risk factors for CHD. These studies further understanding of the effects of ALA intake on haemostasis, endothelial function, immune function, lipoprotein metabolism and its conversion to long chain derivatives.
26. In adults, ALA supplementation causes an increase in the blood and plasma levels of ALA, EPA and DPA but not DHA. Blood and plasma DHA levels are increased by increasing DHA in the diet. It was noted that DHA precursor n-3 PUFA could be transported via the plasma to tissues where subsequent conversion might occur (e.g. myocytes, astrocytes), and **further isotopic studies are needed to investigate other tissue pools of fatty acids.** However, such work is expensive due to the cost of the isotopes.
27. The studies presented as part of this workshop suggest little, if any, benefit of ALA, relative to LA, on risk factors for cardiovascular disease. Effects observed with fish oil supplementation were not replicated by ALA supplementation. No consistent beneficial effects of ALA supplementation were observed on measures

of endothelial function, immune function, serum lipids or measures of haemostatic function; whereas the long chain derivatives of ALA – EPA and DHA – have been demonstrated to have beneficial effects. Although ALA supplementation increases EPA there is no demonstrable benefit on the risk factors investigated in these studies. **Before investigating effects on cardiovascular risk factors any further, it is necessary to establish that ALA consumption has an impact on health outcomes. The panel suggested that there was no reason to look further at effects of ALA supplementation on existing CVD risk factors.**

28. The main cause of sudden death from acute heart attack is ventricular fibrillation. Both clinical and animal studies have suggested that n-3 PUFA have antiarrhythmic effects (Leaf, 1999; Christensen *et al.* 1999, 2000); indeed, the most likely mechanism for the beneficial effect of eicosapentaenoic and docosahexaenoic acid on the recurrence of CHD is the stabilisation of arrhythmias (Marchioli *et al.* 2002). It has also been postulated that this could be an underlying mechanism for the beneficial effect of the Mediterranean ALA-rich diet in the secondary prevention of CHD (de Lorgeril *et al.* 1994, 1999) – either directly or via conversion to EPA (Leaf, 1999; Connor, 1999). The workshop, however, had reservations about the evidence suggesting a beneficial effect of ALA on the secondary prevention of CHD, and felt this still needed to be established. **The panel suggested a randomized clinical trial to investigate the effect of ALA supplementation on the secondary prevention of Coronary Heart Disease.**

Abstracts of the presentations at each of these workshops are available from the SACN secretariat.

**SACN secretariat
June 2002**

References

- Allman-Farinelli, M. A.; Hall, D.; Kingham, K.; Pang, D.; Petocz, P., and Favaloro, E. J. *Comparison of the effects of two low fat diets with different alpha- linolenic:linoleic acid ratios on coagulation and fibrinolysis*. *Atherosclerosis*. 1999; **142**(1):159-68.
- Ascherio, A.; Rimm, E. B.; Giovannucci, E. L.; Spiegelman, D.; Stampfer, M., and Willett, W. C. *Dietary fat and risk of coronary heart disease in men: cohort follow up study in the United States*. *BMJ*. 1996; **313**(7049):84-90.
- Berry, E. M.; Eisenberg, S.; Haratz, D.; Friedlander, Y.; Norman, Y.; Kaufmann, N. A., and Stein, Y. *Effects of diets rich in monounsaturated fatty acids on plasma lipoproteins--the Jerusalem Nutrition Study: high MUFAs vs high PUFAs*. *Am J Clin Nutr*. 1991; **53**(4):899-907.
- Calder, P. C. *N-3 polyunsaturated fatty acids, inflammation and immunity: pouring oil on troubled waters or another fishy tale?* *Nutr Res*. 2001; **21**:309-341.
- Caughey, G. E.; Mantzioris, E.; Gibson, R. A.; Cleland, L. G., and James, M. J. *The effect on human tumor necrosis factor alpha and interleukin 1 beta production of diets enriched in n-3 fatty acids from vegetable oil or fish oil*. *Am J Clin Nutr*. 1996; **63**(1):116-22.
- Christensen, J. H.; Christensen, M. S.; Dyerberg, J., and Schmidt, E. B. *Heart rate variability and fatty acid content of blood cell membranes: a dose-response study with n-3 fatty acids*. *Am J Clin Nutr*. 1999; **70**(3):331-Christensen, J. H.; Christensen, M. S.; Toft, E.; Dyerberg, J., and Schmidt, E. B. *Alpha-linolenic acid and heart rate variability*. *Nutr Metab Cardiovasc Dis*. 2000; **10**(2):57-61.
- Clarke, R.; Frost, C.; Collins, R.; Appleby, P., and Peto, R. *Dietary lipids and blood cholesterol: quantitative meta-analysis of metabolic ward studies*. *BMJ*. 1997; **314**(7074):112-7.
- Connor, W. E. *Alpha-linolenic acid in health and disease*. *Am J Clin Nutr*. 1999; **69**(5):827-8.
- Connor, W. E. and Connor, S. L. *Should a low-fat, high-carbohydrate diet be recommended for everyone? The case for a low-fat, high-carbohydrate diet*. *N Engl J Med*. 1997; **337**(8):562-3; discussion 566-7.
- de Lorgeril, M.; Renaud, S.; Mamelle, N.; Salen, P.; Martin, J. L.; Monjaud, I.; Guidollet, J.; Touboul, P., and Delaye, J. *Mediterranean alpha-linolenic acid-rich diet in secondary prevention of coronary heart disease*. *Lancet*. 1994; **343**(8911):1454-9.
- de Lorgeril, M.; Salen, P.; Martin, J. L.; Monjaud, I.; Delaye, J., and Mamelle, N. *Mediterranean diet, traditional risk factors, and the rate of cardiovascular complications after myocardial infarction: final report of the Lyon Diet Heart Study*. *Circulation*. 1999; **99**(6):779-85.
- Department for the Environment, Food and Rural Affairs. *The National Food Survey; 2000*. London: HMSO; 2001.
- Department of Health. *Nutritional aspects of cardiovascular disease. Report of the Cardiovascular Review Group Committee on Medical Aspects of Food Policy (reports on health and social subjects: 46)*. London: HMSO; 1994.
- Dolecek, T. A. *Epidemiological evidence of relationships between dietary polyunsaturated fatty acids and mortality in the multiple risk factor intervention trial*. *Proc Soc Exp Biol Med*. 1992; **200**(2):177-82.
- Foley, M.; Ball, M.; Chisholm, A.; Duncan, A.; Spears, G., and Mann, J. *Should mono- or poly-unsaturated fats replace saturated fat in the diet?* *Eur J Clin Nutr*. 1992; **46**(6):429-36.

- Freese, R. and Mutanen, M. *Alpha-linolenic acid and marine long-chain n-3 fatty acids differ only slightly in their effects on hemostatic factors in healthy subjects*. Am J Clin Nutr. 1997; **66**(3):591-8.
- GISSI-Prevenzione Investigators. *Dietary supplementation with n-3 polyunsaturated fatty acids and vitamin E after myocardial infarction: results of the GISSI-Prevenzione trial*. Gruppo Italiano per lo Studio della Sopravvivenza nell'Infarto miocardico. Lancet. 1999; **354**(9177):447-55.
- Harris, W. S. *n-3 fatty acids and serum lipoproteins: human studies*. Am J Clin Nutr. 1997; **65**(5 Suppl):1645S-1654S.
- Healy, D. A.; Wallace, F. A.; Miles, E. A.; Calder, P. C., and Newsholme, P. *Effect of low-to-moderate amounts of dietary fish oil on neutrophil lipid composition and function*. Lipids. 2000; **35**(7):763-8.
- Howard, B. V.; Hannah, J. S.; Heiser, C. C.; Jablonski, K. A.; Paidi, M. C.; Alarif, L.; Robbins, D. C., and Howard, W. J. *Polyunsaturated fatty acids result in greater cholesterol lowering and less triacylglycerol elevation than do monounsaturated fatty acids in a dose-response comparison in a multiracial study group*. Am J Clin Nutr. 1995; **62**(2):392-402.
- Hu, F. B.; Stampfer, M. J.; Manson, J. E.; Rimm, E. B.; Wolk, A.; Colditz, G. A.; Hennekens, C. H., and Willett, W. C. *Dietary intake of alpha-linolenic acid and risk of fatal ischemic heart disease among women*. Am J Clin Nutr. 1999; **69**(5):890-7.
- Katan, M. B.; Grundy, S. M., and Willett, W. C. *Should a low-fat, high-carbohydrate diet be recommended for everyone? Beyond low-fat diets*. N Engl J Med. 1997; **337**(8):563-6; discussion 566-7.
- Kelley, D. S.; Branch, L. B.; Love, J. E.; Taylor, P. C.; Rivera, Y. M., and Iacono, J. M. *Dietary alpha-linolenic acid and immunocompetence in humans*. Am J Clin Nutr. 1991; **53**(1):40-6.
- Keys, A.; Menotti, A.; Karvonen, M. J.; Aravanis, C.; Blackburn, H.; Buzina, R.; Djordjevic, B. S.; Dontas, A. S.; Fidanza, F. and Keys, M. H. *The diet and 15-year death rate in the seven countries study*. Am J Epidemiol. 1986; **124**(6):903-15.
- Knapp, H. R. *Dietary fatty acids in human thrombosis and hemostasis*. Am J Clin Nutr. 1997; **65**(5 Suppl):1687S-1698S.
- Kris-Etherton, P. M.; Pearson, T. A.; Wan, Y.; Hargrove, R. L.; Moriarty, K.; Fishell, V., and Etherton, T. D. *High-monounsaturated fatty acid diets lower both plasma cholesterol and triacylglycerol concentrations*. Am J Clin Nutr. 1999; **70**(6):1009-15.
- Kris-Etherton, P. M.; Pelkman, C. L.; Zhao, G.; Pearson, T. A.; Wan, Y., and Etherton, T. D. *Reply to P marckmann*. Am J Clin Nutr. 2000; **72**(3):854-6.
- Leaf, A. *Dietary prevention of coronary heart disease: the Lyon Diet Heart Study*. Circulation. 1999 **16**; 99(6):733-5.
- Lichtenstein, A. H.; Ausman, L. M.; Carrasco, W.; Jenner, J. L.; Gualtieri, L. J.; Goldin, B. R.; Ordovas, J. M., and Schaefer, E. J. *Effects of canola, corn, and olive oils on fasting and postprandial plasma lipoproteins in humans as part of a National Cholesterol Education Program Step 2 diet*. Arterioscler Thromb. 1993; **13**(10):1533-42.
- Marchioli, R.; Barzi, F.; Bomba, E.; Chieffo, C.; Di Gregorio, D.; Di Mascio, R.; Franzosi, M. G.; Geraci, E.; Levantesi, G.; Maggioni, A. P.; Mantini, L.; Marfisi, R. M.; Mastrogiuseppe, G.; Mininni, N.; Nicolosi, G. L.; Santini, M.; Schweiger, C.; Tavazzi, L.; Tognoni, G.; Tucci, C., and Valagussa, F. *Early protection against sudden death by n-3 polyunsaturated fatty acids after myocardial infarction: time-course analysis of the*

results of the Gruppo Italiano per lo Studio della Sopravvivenza nell'Infarto Miocardico (GISSI)-Prevenzione. Circulation. 2002; **105**(16):1897-903.

Marckmann, P. and Astrup, A. *Fatty diets are unhealthy--even those based on monounsaturates.* Am J Clin Nutr. 2000; **72**(3):853-6.

Mata, P.; Garrido, J. A.; Ordoñas, J. M.; Blázquez, E.; Alvarez-Sala, L. A.; Rubio, M. J.; Alonso, R., and de Oya, M. *Effect of dietary monounsaturated fatty acids on plasma lipoproteins and apolipoproteins in women.* Am J Clin Nutr. 1992; **56**(1):77-83.

McDonald, B. E.; Gerrard, J. M.; Bruce, V. M., and Corner, E. J. *Comparison of the effect of canola oil and sunflower oil on plasma lipids and lipoproteins and on in vivo thromboxane A2 and prostacyclin production in healthy young men.* Am J Clin Nutr. 1989; **50**(6):1382-8.

Mensink, R. P. and Katan, M. B. *Effect of a diet enriched with monounsaturated or polyunsaturated fatty acids on levels of low-density and high-density lipoprotein cholesterol in healthy women and men.* N Engl J Med. 1989; **321**(7):436-41.

Mensink, R. P. and Katan, M. B. *Effect of dietary fatty acids on serum lipids and lipoproteins. A meta-analysis of 27 trials.* Arterioscler Thromb. 1992; **12**(8):911-9.

Mensink, R. P. and Katan, M. B. *Effect of monounsaturated fatty acids versus complex carbohydrates on high-density lipoproteins in healthy men and women.* Lancet. 1987; **1**(8525):122-5.

Miller, G. J.; Cruickshank, J. K.; Ellis, L. J.; Thompson, R. L.; Wilkes, H. C.; Stirling, Y.; Mitropoulos, K. A.; Allison, J. V.; Fox, T. E., and Walker, A. O. *Fat consumption and factor VII coagulant activity in middle-aged men. An association between a dietary and thrombogenic coronary risk factor.* Atherosclerosis. 1989; **78**(1):19-24.

Mutanen, M. and Freese, R. *Fats, lipids and blood coagulation.* Curr Opin Lipidol. 2001; **12**(1):25-9.

National Cholesterol Education Program. *Executive Summary of The Third Report of The National Cholesterol Education Program (NCEP) Expert Panel on Detection, Evaluation, And Treatment of High Blood Cholesterol In Adults (Adult Treatment Panel III).* JAMA. 2001; **285**(19):2486-97.

Nestel, P. J. *Fish oil and cardiovascular disease: lipids and arterial function.* Am J Clin Nutr. 2000; **71**(1 Suppl):228S-31S.

Nestel, P. J.; Pomeroy, S. E.; Sasahara, T.; Yamashita, T.; Liang, Y. L.; Dart, A. M.; Jennings, G. L.; Abbey, M., and Cameron, J. D. *Arterial compliance in obese subjects is improved with dietary plant n-3 fatty acid from flaxseed oil despite increased LDL oxidizability.* Arterioscler Thromb Vasc Biol. 1997; **17**(6):1163-70.

Oomen, C. M.; Ocke, M. C.; Feskens, E. J.; Kok, F. J., and Kromhout, D. *alpha-Linolenic acid intake is not beneficially associated with 10-y risk of coronary artery disease incidence: the Zutphen Elderly Study.* Am J Clin Nutr. 2001; **74**(4):457-63.

Pang, D.; Allman-Farinelli, M. A.; Wong, T.; Barnes, R., and Kingham, K. M. *Replacement of linoleic acid with alpha-linolenic acid does not alter blood lipids in normolipidaemic men.* Br J Nutr. 1998; **80**(2):163-7.

Roche, H. M.; Zampelas, A.; Knapper, J. M.; Webb, D.; Brooks, C.; Jackson, K. G.; Wright, J. W.; Gould, B. J.; Kafatos, A.; Gibney, M. J., and Williams, C. M. *Effect of long-term olive oil dietary intervention on postprandial triacylglycerol and factor VII metabolism.* Am J Clin Nutr. 1998; **68**(3):552-60.

Schaefer, E. J. *Lipoproteins, nutrition, and heart disease.* Am J Clin Nutr. 2002; **75**(2):191-212.

- Sirtori, C. R.; Gatti, E.; Tremoli, E.; Galli, C.; Gianfranceschi, G.; Franceschini, G.; Colli, S.; Maderna, P.; Marangoni, F. and Perego, P. *Olive oil, corn oil, and n-3 fatty acids differently affect lipids, lipoproteins, platelets, and superoxide formation in type II hypercholesterolemia*. Am J Clin Nutr. 1992; **56**(1):113-22.
- Thies, F.; Miles, E. A.; Nebe-von-Caron, G.; Powell, J. R.; Hurst, T. L.; Newsholme, E. A., and Calder, P. C. *Influence of dietary supplementation with long-chain n-3 or n-6 polyunsaturated fatty acids on blood inflammatory cell populations and functions and on plasma soluble adhesion molecules in healthy adults*. Lipids. 2001; **36**(11):1183-93.
- Thies, F.; Nebe-von-Caron, G.; Powell, J. R.; Yaqoob, P.; Newsholme, E. A., and Calder, P. C. *Dietary supplementation with eicosapentaenoic acid, but not with other long-chain n-3 or n-6 polyunsaturated fatty acids, decreases natural killer cell activity in healthy subjects aged >55 y*. Am J Clin Nutr. 2001; **73**(3):539-48.
- Thies, F.; Nebe-von-Caron, G.; Powell, J. R.; Yaqoob, P.; Newsholme, E. A., and Calder, P. C. *Dietary supplementation with gamma-linolenic acid or fish oil decreases T lymphocyte proliferation in healthy older humans*. J Nutr. 2001; **131**(7):1918-27.
- Wahrburg, U.; Martin, H.; Sandkamp, M.; Schulte, H., and Assmann, G. *Comparative effects of a recommended lipid-lowering diet vs a diet rich in monounsaturated fatty acids on serum lipid profiles in healthy young adults*. Am J Clin Nutr. 1992; **56**(4):678-83.
- Williams, C. M.; Francis-Knapper, J. A.; Webb, D.; Brookes, C. A.; Zampelas, A.; Tredger, J. A.; Wright, J.; Meijer, G.; Calder, P. C.; Yaqoob, P.; Roche, H., and Gibney, M. J. *Cholesterol reduction using manufactured foods high in monounsaturated fatty acids: a randomized crossover study*. Br J Nutr. 1999; **81**(6):439-46.